

Estimating diagnostic test accuracy for infectious salmon anaemia virus in Maine, USA

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Abstract

Infectious salmon anaemia virus (ISAV) is a pathogen of consequence to farmed Atlantic salmon, *Salmo salar* L. ISA control centres on active surveillance for early detection by reverse transcription polymerase chain reaction (RT-PCR), indirect fluorescent antibody assay (IFAT) and virus isolation. Because diagnostic test performance varies among populations and laboratories, the Office International des Epizooties (OIE) recommends an evaluation of test accuracy in each region of use. This is complicated in Maine, USA by the co-existence of ISAV genotypes homologous to North American (NA) and European (EU) isolates. While NA ISAV genotypes isolated in Maine are characterized by high morbidity and mortality, the single EU genotype in Maine has not yet been linked to disease or isolated by cell culture. Consequently, distinguishing among genotypes is critical to infection response. Accuracy in NA genotype detection was estimated from ISA surveillance data using latent class models. Results suggested that RT-PCR is an excellent screening test for NA ISAV genotypes in Maine, although probably with reduced specificity in the presence of EU genotypes. IFAT, in contrast, was a poor screening test for detection of ISAV in Maine, although it may be useful in confirmation of NA genotypes during disease outbreaks.

Keywords: IFAT, infectious salmon anaemia, RT-PCR, *Salmo salar*, sensitivity, specificity.

Introduction

Outbreaks of disease caused by infectious salmon anaemia virus (ISAV), an orthomyxovirus specific to salmonids (Mjaaland, Rimstad, Falk & Dannevig 1997; Krossoy, Hordvik, Nilsen, Nylund & Endresen 1999), have caused severe morbidity and mortality in farmed Atlantic salmon, *Salmo salar* L., in Maine, USA (Bouchard, Brockway, Giray, Keleher & Merrill 2001), New Brunswick, Canada (Mullins, Groman & Wadowska 1998), Norway (Thorud & Djupvik 1988), the United Kingdom (Rodger, Turnbull, Muir, Millar & Richards 1998) and the Faroe Islands (Anonymous 2000). In December 2001, the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) coordinated with Maine's Department of Marine Resources (DMR) and the local aquaculture industry in the formation of an ISA programme to minimize the spread of, and the losses attributed to, ISAV (USDA APHIS Veterinary Services, Maine Department of Marine Resources and Maine Aquaculture Association 2002). Active surveillance to achieve early detection and prompt removal of infected cages is a central feature of the ISA programme in Maine.

Active surveillance for ISA includes minimum monthly veterinary inspections, and testing of selected moribund fish, at all operating Atlantic salmon farms in Maine. All surveillance specimens are individually tested by reverse transcription

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polymerase chain reaction (RT-PCR) and an indirect fluorescent antibody assay (IFAT). Virus isolation by cell culture is also performed on pooled specimens collected during follow-up visits to confirm positive laboratory results or to investigate sites with elevated risks of ISA disease. Because a gold standard test for ISAV with known and exceptional sensitivity and specificity is not available, Atlantic salmon cages are deemed infected based on a combination of test results. Two fish from a specific cage found ISAV positive by at least two tests is the threshold for a determination of ISAV infection, and mandatory depopulation of the affected cage. Depopulation is initiated, either voluntarily by the industry or under mandate by the State of Maine, within 15 days of virus confirmation (USDA APHIS Veterinary Services, Maine Department of Marine Resources and Maine Aquaculture Association 2002).

Studies from neighbouring New Brunswick, Canada report ISAV diagnostic test sensitivity and specificity, respectively, at 0.93 and 0.98 for RT-PCR (McClure, Hammell, Stryhn & Dohoo 2005) and 0.79 and 0.96 for IFAT (with a cut-off between 0 and 1+) (McClure *et al.* 2005). Ring testing is conducted annually to minimize differences among the various laboratories responsible for ISAV testing in Atlantic Canada and Maine. However, because test performance is also affected by population-specific parameters, such as stage and severity of disease, virus genotype and the presence of cross-reactors (Greiner & Gardner 2000), the World Organization for Animal Health [Office International des Epizooties (OIE)] recommends a separate evaluation of diagnostic test sensitivity and specificity in each region and context of interest (Office International des Epizooties 2003).

Calculation of diagnostic test sensitivity and specificity in Maine is complicated by the presence of multiple genotypes of ISAV. Efforts to classify ISAV genotypes worldwide have established two divergent branches in the phylogenetic tree (Blake, Bouchard, Keleher, Opitz & Nicholson 1999; Krossoy, Nilsen, Falk, Endresen & Nylund 2001; Mjaaland, Hungnes, Teig, Dannevig, Thorud & Rimstad 2002; Nylund, Devold, Plarre, Isdal & Aarseth 2003). The North American (NA) branch characterizes the majority of virus isolates found in Canada and the USA, while those from Scandinavia and the British Isles are grouped under the European (EU) branch. The predominant highly polymorphic region (HPR)21 (Nylund *et al.* 2003)

genotypes found to date in Maine, HPR21, HPR21a and HPR21b (C. Giray, personal communication; Warg, Ellis, Gustafson, Robinson, Marengi & Giray 2006), have been associated with disease and fall into the NA cluster (USDA APHIS VS ISA Programme, unpublished data). These virulent genotypes, detectable by both RT-PCR and IFAT, are associated with clinical signs and escalating mortality in fish on affected farms (USDA APHIS VS ISA programme, unpublished data) and are readily isolated in cell culture using CHSE-214 (Lannan, Winton & Fryer 1984; Bouchard, Keleher, Opitz, Blake, Edwards & Nicholson 1999; Clouthier, Rector, Brown & Anderson 2002), SHK (Dannevig, Brudeseth, Gjoen, Rode, Nergeland, Evensen & Press 1997) and ASK cell lines (Rolland, Bouchard, Coll & Winton 2005).

Concurrently, since 2003, an ISAV genotype homologous with EU isolates has also been detected in Maine (Giray *et al.* GenBank accessions AY575955 and AY534683; Plarre *et al.* GenBank accession AY973194; C. Giray, personal communication). Similar reports originated from New Brunswick (Cook-Versloot, Griffiths, Cusack, McGeachy & Ritchie 2004) and earlier from Nova Scotia, Canada (Ritchie, Cook, Melville, Simard, Cusack & Griffiths 2001). This genotype has a much different diagnostic and clinical presence than other genotypes found in Maine. Confirmed as genotype HPR0 by sequencing of the HPR of ISAV genome segment 6, this genotype has only been consistently identified by RT-PCR and is not readily detected by standard IFAT or virus isolation techniques (C. Giray, personal communication). Additionally, the HPR0 genotype has not, to date, been associated with clinical signs or ISA-attributed mortality in farmed Atlantic salmon in Maine (USDA APHIS VS ISA Programme, unpublished data). A single geographically isolated farm in Maine showed a rise and fall in ISAV HPR0 genotype detections over time (USDA APHIS VS ISA Programme, unpublished data), although sampling intensity may explain some of this variation. However, occurrences of the HPR0 genotype on most farms in Maine appear sporadic and self limited. Because this genotype has not yet caused clinical signs, nor met the 2-fish-positive-by-2-tests criterion for cage-level depopulation, its occurrence on a site is of lesser immediate consequence to the industry. This is in stark contrast to the rapid and drastic action undertaken through the depopulation of an entire cage (averaging 10 000–30 000 fish), in

response to confirmation of infection by the HPR21 (NA) genotypes. As a result, distinguishing between genotypes has become a priority of the ISA surveillance programme. Diagnostic tests employed by any ISA surveillance programme now need to address two goals: (1) detection of the presence of ISAV and (2) differentiation between genotypes relative to disease.

Sensitivity and specificity can be estimated directly by comparing test results to 'true' disease status as determined by a gold standard test. In the absence of a gold standard, however, sensitivity and specificity are estimated using latent-class models based on maximum-likelihood or Bayesian inference techniques (Enoe, Georgiadis & Johnson 2000). In these models, sensitivity and specificity are derived from the results of two parallel diagnostic tests from two or more populations of differing disease prevalence. Designating prevalence populations for ISAV genotypes associated with disease is entirely feasible in Maine. Local outbreaks of ISA disease in the past have centred in Cobscook and Passamaquoddy Bays in the far eastern part of the state adjoining Canadian marine waters (Fig. 1). Local distribution of the HPR0 genotype, in contrast, is more widely distributed geographically and appears to differ more by season than

by location, with limited detections in warmer months.

Using ISA Programme data from 2002 to 2005, we evaluated sensitivity and specificity of ISAV screening tests in two ways. First, we performed TAGS (Tests in the Absence of a Gold Standard) analyses on the full dataset to estimate RT-PCR and IFAT performance for the detection of NA and EU genotypes separately. Second, using the subset of RT-PCR positive submissions that were sequenced for genotype determination, we calculated a direct estimate (using genotype results as a gold standard) of the accuracy of IFAT in differentiating NA from HPR0 genotypes among RT-PCR test positive samples.

Materials and methods

Surveillance data

Under the ISA surveillance programme, all active Atlantic salmon farms in Maine were inspected at least monthly by a licensed and USDA-APHIS accredited veterinarian, increasing to weekly or biweekly for farms deemed high risk by disease history, clinical findings or suspect laboratory results. Veterinary inspections included observation of all

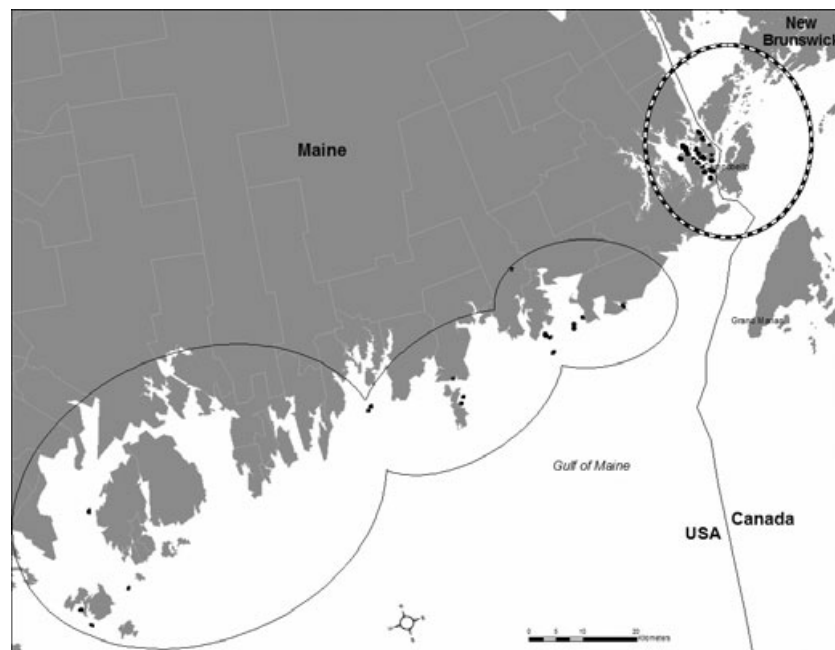


Figure 1 Map showing the distribution of Atlantic salmon farms in Maine, USA that were active during 2002–2005. Farm sites are shown as polygons. The high-risk region for ISAV occurrence is circled by a dark dashed line. The low-risk region is circled by a thin solid line. The international boundary between Maine, USA and New Brunswick, Canada is also displayed.

cages for abnormal or slow-swimming fish, discussion of morbidity and mortality issues with site managers and divers, and targeted selection of 10–30 moribund fish (or fresh mortalities) by dip-net or diver for lethal collection and submission of tissues for ISAV testing (USDA APHIS Veterinary Services, Maine Department of Marine Resources and Maine Aquaculture Association 2002). Apparently healthy fish, amenable to capture, were occasionally collected when moribund fish were unavailable. The resulting study design from this surveillance system was cross-sectional and systematic at the level of the farms, and targeted to moribund animals at the level of the fish. The minimum sampling frequency and uniform response to suspect findings ensured that all farms had an equal chance of baseline representation in the study. All surveillance laboratory assays were conducted by Micro Technologies, Inc. (Richmond, ME, USA), with confirmatory testing of initial positives carried out by USDA APHIS National Veterinary Services Laboratories (NVSL, Ames, IA, USA). All results from RT-PCR and IFAT tests run in parallel, from surveillance samples submitted from Atlantic salmon farms in Maine during 2002–2005, were included in the study. Virus isolation, conducted on a subset of submissions, was often run on pooled samples and so was not considered further in this study.

RT-PCR

RNA was extracted using the RNeasy MiniKit (Qiagen, Valencia, CA, USA) from 20 to 30 mg of middle kidney tissue preserved in RNAlater®. To account for RNA yield variation between samples, extracts were spectrophotometrically quantified and 50–100 ng of total RNA was inoculated per reaction. Amplification was performed using the 1D/2 primer set (Blake *et al.* 1999) which amplifies a 493 base pair region of ISAV genome segment 8. Each new primer shipment was analysed using dilutions of re-hydrated primer preparations and RNA to standardize the amount used for optimal reaction yields. The total used per 25 µL reaction varied between 12.5 and 25 pmol of each primer. The GeneAmp EZ rTth RNA PCR Kit (Roche Applied Biosystems, Foster City, CA, USA) was used for amplification as follows: 60 °C for 30 min, 94 °C for 1 min, 40 cycles of 94 °C for 20 s and 59 °C for 40 s, followed by a 7 min extension at 59 °C. RT-PCR products were electrophoresed alongside a 100 base pair DNA ladder and ISAV-

positive tissue control on a 10 cm 2% agarose gel at 60 V for 80 min. Gels were stained with SYBR Green for 30–40 min and photographed under UV illumination. The observation of a 493 base pair amplified band was considered an ISAV-positive result and confirmed by re-amplification of the original RNA extract as well as a fresh extract from the preserved tissue sample. Analysis of RT-PCR positive results by follow-up sequencing of segment 8 and/or 6 was used to confirm and characterize ISAV isolates on a cage-by-cage basis.

IFAT

Testing by IFAT was performed using mid-kidney slide impressions and monoclonal antibody to ISAV (Falk, Namork & Dannevig 1998) using the technique outlined by the Office International des Epizooties (2003). IFAT slides were scored from 0 to 4+, based on staining patterns, fluorescence intensity and the number of fluorescing cells viewed per field. IFAT ratings of 3+ and above were accepted as an ISAV-positive result.

Screening test performance

Estimation of RT-PCR and IFAT accuracy in the detection of NA ISAV genotypes in Maine was based on three prevalence populations. Experience of previous years distinguished the Cobscook and Passamaquoddy Bay regions of Maine as high risk for ISA disease, relative to the rest of the coast where clinical infection had not yet been observed (Fig. 1). The surveillance samples from this high-risk region were further grouped into medium and high prevalence, based on the distinction between first and second year fish (referring to the number of years in the marine environment), respectively, with an April 1 cut-off. Observations from surveillance programme data suggest that fish are more likely to become infected with ISAV after their first year in sea water (USDA APHIS VS ISA Programme, unpublished data). This provided three populations of differing prevalence for the study: high (second year) and medium (first year) fish in Cobscook/Passamaquoddy Bays, and low (all year classes) elsewhere in Maine (Fig. 1).

Test performance was evaluated separately for NA vs. EU genotypes, i.e. those associated and not associated with disease, respectively, based on their observed spatial and temporal distributions. Specificity and sensitivity for RT-PCR and IFAT

screening tests were estimated in the absence of a gold standard using the 'TAGS' programme (<http://www.epi.ucdavis.edu/diagnostictests/>) for maximum-likelihood estimation (Enoe *et al.* 2000). This programme simultaneously solves multiple equations (prevalence among each population, and sensitivity and specificity of each test) to generate estimates. The TAGS analysis was run twice: (1) using prevalence populations that followed the spatial distribution of genotypes associated with disease and (2) using prevalence populations that followed the temporal distribution of the genotype not associated with disease. However, the EU evaluation was not completed due to apparent assumption violations detected during analysis.

Genotype differentiation by IFAT

Sensitivity and specificity of IFAT for distinguishing between NA and EU RT-PCR positives was evaluated from the subset of samples with concurrent genotype results, using sequencing as a gold standard. 95% confidence intervals for sensitivity and specificity proportions were calculated according to the efficient-score method (Newcombe 1998) using the Vassar Stats on-line calculator (<http://faculty.vassar.edu/lowry/clin1.html>). Sensitivity and specificity was estimated for each of the possible positive threshold values for IFAT (i.e. $\geq 1+$, $\geq 2+$, $\geq 3+$, $\geq 4+$).

Results

Surveillance results

During 2002–2005, the USDA APHIS VS ISA Surveillance Programme ran parallel RT-PCR and

IFAT tests on 6435 Atlantic salmon from farms in Cobscook or Passamaquoddy Bays, and another 4026 salmon from farms outside these bays. Of these 10 461 total submissions, 458 were determined to be positive by RT-PCR and 78 by IFAT (based on a rating of 3+ or 4+). A subset (180) of those found positive by RT-PCR was analysed for genotype determination by sequencing of the targeted portion of the genome (typically the first positive RT-PCR result in a given cage). Sequencing found 138 of the NA and 42 of the EU genotype. One submission sequenced both NA and EU genotypes. The NA genotypes were detected year-round (Fig. 2) and were concentrated in the Cobscook/Passamaquoddy Bay regions. The EU genotype did not centre in any one geographic region, but showed a seasonal pattern with complete cessation of detections during July to September (Fig. 2).

Screening test performance

TAGS evaluation of test performance for NA genotypes was based on 4026 samples from a low prevalence population, 2676 samples from a medium prevalence population and 3759 samples from a high prevalence population (Table 1). A TAGS evaluation of test performance with NA genotypes estimated sensitivity and specificity of 89.2% and 98.7% for RT-PCR, and 18.4% and 99.9% for IFAT, respectively, at a rating of 3+ or higher (Table 2). TAGS evaluation of test performance for EU genotypes was based on 2871 samples from a high prevalence population, 7228 samples from a medium prevalence population and 362 samples from a low prevalence population

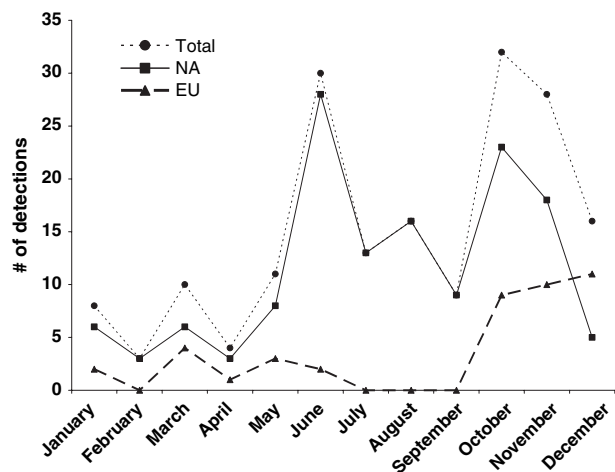


Figure 2 Number of ISAV genotype detections per sample month for 2002–2005. NA, North American; EU, European.

Table 1 Summary data for TAGS evaluation of RT-PCR and IFAT sensitivity and specificity to North American genotypes of ISAV. The high prevalence population includes fish collected in Cobscook and Passamaquoddy Bays (Maine) from the April after each new year class stocking, until harvest or the end of 2005. The medium prevalence population includes fish collected in Cobscook and Passamaquoddy Bays from stocking of each new year class, until the end of the following March. The low prevalence population includes fish collected from sites in Maine outside Cobscook and Passamaquoddy

	High prevalence			Medium prevalence			Low prevalence		
IFAT	≥3+	<3+	Total	≥3+	<3+	Total	≥3+	<3+	Total
PCR+	55	282	337	4	64	68	0	53	53
PCR–	11	3411	3422	2	2606	2608	6	3967	3973
Total	66	3693	3759	6	2670	2676	6	4019	4026

Table 2 Estimates for sensitivity and specificity for RT-PCR and IFAT for detection of North American genotypes of ISAV found in farmed Atlantic salmon in Maine 2002–2005. Lower and upper CI refers to the bounds of the 95% confidence interval

	Se	Lower CI	Upper CI	Sp	Lower CI	Upper CI
RT-PCR	0.8915	0.7311	0.9613	0.9863	0.9823	0.9894
IFAT	0.1844	0.1431	0.2343	0.9988	0.9975	0.9994

(Table 3). A TAGS evaluation of sensitivity and specificity for the EU genotype, based on seasonal distributions, was attempted. However, the model detected apparent assumption violations, so test performance characteristics for the EU genotype were not calculated.

Genotype differentiation by IFAT

The accuracy of IFAT as a confirmatory test for NA genotypes (to differentiate from positive RT-PCR results caused by the EU genotype) was explored by reviewing its performance among the subset of RT-PCR positive samples of known genotype. Using sequence classifications (NA vs. EU) as a gold standard (summary data presented in Table 4), the sensitivity and specificity of IFAT for differentiation of the two genotypes was found to be 16.3% and 97.7%, respectively, at a cut-off of 3+ (Table 5). Lower cut-off values decreased specificity and

Table 4 Summary data for the evaluation of sensitivity and specificity of IFAT for distinguishing between North American (NA) and European (not NA) genotypes of ISAV. Includes only the RT-PCR positives that have been sequenced (does not include bandwidth comparison)

	≥1+	<1+	≥2+	<2+	≥3+	<3+	≥4+	<4+	Total
NA	57	81	39	99	22	116	3	135	138
Not NA	4	38	3	39	1	41	0	42	42
Total	61	119	42	138	23	157	3	177	180

Table 5 Estimates for the sensitivity and specificity of IFAT for distinguishing between North American and European genotypes of ISAV. Estimates are based on sequenced samples from farmed Atlantic salmon in Maine 2002–2005. Lower and upper CI refers to the bounds of the 95% confidence interval

	Se	Lower CI	Upper CI	Sp	Lower CI	Upper CI
IFAT ≥4	0.0217	0.0056	0.0671	1	0.8956	1
IFAT ≥3	0.1594	0.1047	0.2336	0.9762	0.8591	0.9988
IFAT ≥2	0.2826	0.2109	0.3666	0.9286	0.7945	0.9814
IFAT ≥1	0.4130	0.3309	0.5000	0.9048	0.7645	0.9690

higher cut-off values decreased sensitivity substantially (Table 5).

Discussion

The diagnostic test accuracy of the RT-PCR assay targeting a conserved region of ISAV segment 8 was

Table 3 Summary data for TAGS evaluation of RT-PCR and IFAT sensitivity and specificity to the European genotype of ISAV. The high prevalence includes fish collected from a site with a distinct winter/spring cluster of European genotype detections. The medium prevalence population includes fish taken from October to June of each year (excluding the high prevalence population above). The low prevalence population includes fish collected from July to September of each year

	High prevalence			Medium prevalence			Low prevalence		
IFAT	≥3+	<3+	Total	≥3+	<3+	Total	≥3+	<3+	Total
PCR+	0	40	40	46	239	285	13	120	133
PCR–	3	319	322	8	6935	6943	8	2730	2738
Total	3	359	362	54	7174	7228	21	2850	2871

highly sensitive to the NA ISAV genotypes found in Maine during 2002–2005. This RT-PCR assay was also able to detect a EU genotype not associated with disease but present in the same marine waters. This concurrence explains a slight limitation in assay specificity for the predominant NA genotypes in Maine. The ISAV IFAT, in contrast, demonstrates only low sensitivity, but very high specificity, for the NA genotypes found in Maine. The low sensitivity of the IFAT severely limits its utility as a screening test for ISAV. However, its high specificity suggests that the IFAT may be useful for confirmatory purposes, although such use would presumably still produce many false-negative results.

Population level factors, such as stage and duration of disease, age and condition of animals, and presence of cross-reacting substances, can affect test accuracy (Greiner & Gardner 2000). Consequently, surveys of non-homogeneous populations may produce estimates that are biased towards the most heavily sampled (but not necessarily representative) subsets of the group. Although our study included multiple farms (12–22 per year) in multiple years, our target population and sampling strategy can be considered relatively homogeneous. Most farms operated under fairly uniform standards and conditions during the study period. Industry consolidation, experience with previous disease outbreaks, efforts to respond appropriately to public concerns (and resulting regulations) relating to environmental and endangered species status, active monitoring for the efficacy and safety of an Investigational New Animal Drug (INAD) against sea lice (Gustafson, Ellis, Robinson, Marengi & Endris 2006), and adoption of the ISA Programme Standards (USDA APHIS Veterinary Services, Maine Department of Marine Resources and Maine Aquaculture Association 2002) led to farms with similar stocking, husbandry and disease prevention and response practices. All farms were sampled with equal baseline intensity (minimum 10 fish per month). Farms with apparent or suspect disease were sampled more frequently (weekly or biweekly, rather than monthly), although all were subject to the same risk-based inspection criteria. However, a consequent bias due to non-proportional sampling of clinically suspect farms could result. Likewise, because the surveillance programme targeted moribund fish, the reported estimates of test performance are most representative of similarly targeted sampling strategies.

TAGS makes two assumptions: (1) independence of test results conditional on infection status and (2) constant test sensitivity and specificity across all populations. The former assumption is probably upheld as the two testing modalities use entirely different targets: RT-PCR targets viral RNA, while IFAT targets viral surface proteins. The second assumption may be more problematic. Test performance may vary with the stage of disease and presence of genetically related viruses. Without additional sequence analysis, the EU genotype can be mistaken in diagnostic tests for the NA genotypes and *vice versa*. Because the latter are by far the predominant genotypes detected in Maine, and because their patterns of occurrence vary dramatically by region, it is easy to see how their presence might heavily influence estimates of test performance for the less-prevalent EU genotype. Consequently, specificity of RT-PCR for the EU genotype might be fairly high in regions free of disease caused by ISAV, but exceptionally low in regions experiencing an outbreak where most virus detections will be of the NA genotypes (in this case considered a false positive). Violation of either assumption can lead to bias and reduced accuracy of sensitivity and specificity estimates. For this reason, we did not include results from our TAGS analysis for the EU genotype. In contrast, the TAGS programme output for sensitivity and specificity did not question model assumptions for the NA genotype.

Earlier work targeting Canadian laboratory data (McClure *et al.* 2005) reported substantially higher sensitivities for IFAT tests and lower specificities for RT-PCR. This may be due, in part, to differences in laboratory protocols and/or to differences in cut-off thresholds used for IFAT interpretation. The USDA ISA Programme uses RT-PCR and IFAT to screen all samples, and acts on a 3+ or 4+ IFAT rating as a positive result, with 2+ as a suspect. The New Brunswick ISA Programme, in contrast, frequently uses the IFAT as a primary screening tool, and acts on 2+ or higher rating, with 1+ considered suspect. The seeming discrepancy in test accuracy may also be an artifact of differing study designs. Our surveillance data ranged in ISAV infection and exposure from none to mild/moderate to severe. In contrast, McClure *et al.* (2005) selected samples from known positive sites (based on initial test results correlating with strong clinical signs and elevated mortality), and known negative sites (i.e. ISA detection-free sites), but did not include fish from sites that were subclinical or not

readily categorized. Exclusion of subclinical sites, typically the most difficult to diagnose, could conceivably elevate calculations of test sensitivity (Greiner & Gardner 2000).

This study provides evidence to support the continued use of RT-PCR, with both high sensitivity and high specificity, as a reliable screening test for surveillance for ISAV. The reliability of the IFAT as a screening test, in contrast, is questioned by its low general sensitivity. Its very high specificity for NA genotypes, however, suggests that the IFAT might be better used as a confirmatory test, to differentiate between genotypes for samples found positive by RT-PCR.

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